

PATENT

Case 2713/12

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN RE APPLICATION OF: |

S. C. Bauer et al. | GROUP ART UNIT: not assigned

5 SERIAL NUMBER: not assigned | EXAMINER: not assigned

FILED: March 4, 2002 | DATE: March 4, 2002

TITLE: Methods Of Ex-Vivo Expansion Of Hematopoietic Cells Using Interleukin-3 (IL-3) Multiple Mutation Polypeptides

10 **PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.111**

Assistant Commissioner of Patents  
Washington, D. C. 20231

Sir:

15 Submitted herewith is a Preliminary Amendment under 37 C.F.R. § 1.111. Please amend the application as follows:

**IN THE SPECIFICATION**

20 Please replace the first line of the specification with the following.

-- This is a divisional of United States Serial No. 08/764,114; which was filed December 09, 1996, pending; which a continuation-in-part of United States Serial No. 08/411,795, filed April 06, 1995, now U.S. Patent No. 5,604,116, said 08/411,795 was filed under 35 U.S.C. § 371 from PCT/US93/11197, filed November 22, 1993; which is continuation-in-part of United States Serial No. 07/981,044 filed November 24, 1992, now abandoned. The noted applications are incorporated herein by reference. --

30 **IN THE CLAIMS**

Please cancel claims 1-26 without prejudice.

35 Please add the following new claims 27-34.

-- 27. Cultured stem cells obtained from a method of ex vivo expansion of stem cells, comprising the steps of;

(a) culturing stem cells with a growth medium comprising a human interleukin-3 mutant polypeptide selected from the group consisting of:

- 5 (i) the human interleukin-3 mutant polypeptide of (SEQ ID NO:15);

wherein Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

- 10 Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;  
 Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;  
 Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;  
 Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;
- 15 Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val or Gly;  
 Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Ser, or Arg;  
 Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;
- 20 Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;  
 Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;  
 Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;  
 Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;  
 Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;
- 25 Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;  
 Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;  
 Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;  
 Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;
- 30 Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;  
 Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;  
 Xaa at position 36 is Asp, Leu, or Val;  
 Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
- 35 Xaa at position 38 is Asn, or Ala;  
 Xaa at position 40 is Leu, Trp, or Arg;  
 Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;  
 Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;

- Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;
- Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Asn, Gln, Ala or Pro;
- 5 Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;
- Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;
- Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;
- 10 Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;
- Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
- Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;
- 15 Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
- Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or Met;
- Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;
- 20 Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;
- Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;
- Xaa at position 57 is Asn or Gly;
- 25 Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
- Xaa at position 59 is Glu, Tyr, His, Leu, Pro, or Arg;
- Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;
- Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
- Xaa at position 62 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
- 30 Xaa at position 63 is Arg, Tyr, Trp, Ser, His, Pro, or Val;
- Xaa at position 64 is Ala, Asn, Pro, Ser, or Lys;
- Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
- Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
- Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
- 35 Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
- Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
- Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
- 40 Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;

Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;  
 Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;  
 Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;  
 Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,  
 5           Gln, or Leu;  
 Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or  
           Asp;  
 Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;  
 Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;  
 10   Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or  
           Asp;  
 Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;  
 Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;  
 Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His,  
 15           Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;  
 Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;  
 Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;  
 Xaa at position 85 is Leu, Asn, Val, or Gln;  
 Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;  
 20   Xaa at position 87 is Leu, Ser, Trp, or Gly;  
 Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;  
 Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or  
           Ser;  
 Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;  
 25   Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;  
 Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile  
           or Leu;  
 Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;  
 Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His,  
 30           Ala, or Pro;  
 Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn,  
           Lys, Ser, Ala, Trp, Phe, Ile, or Tyr;  
 Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;  
 Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;  
 35   Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr,  
           Glu, Gln, Ser, Phe, Met, Val, Lys, Tyr or Pro;  
 Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln,  
           Gly, Ser, Phe, or His;  
 Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln,  
 40           or Pro;  
 Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,

Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;  
 Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;  
 Xaa at position 103 is Asp, or Ser;  
 Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu,  
 5 Gln, Lys, Ala, Phe, or Gly;  
 Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,  
 Leu, Lys, Ile, Asp, or His;  
 Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;  
 Xaa at position 108 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His,  
 10 Ser, Ala or Pro;  
 Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;  
 Xaa at position 110 is Lys, Ala, Asn, Thr, Leu, Arg, Gln, His,  
 Glu, Ser, or Trp;  
 Xaa at position 111 is Leu, Ile, Arg, Asp, or Met;  
 15 Xaa at position 112 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;  
 Xaa at position 113 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp,  
 Lys, Leu, Ile, Val or Asn;  
 Xaa at position 114 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;  
 Xaa at position 115 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,  
 20 Trp, or Met;  
 Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu,  
 Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;  
 Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;  
 Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;  
 25 Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;  
 Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;  
 Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or  
 Gly;  
 Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,  
 30 Ile, Tyr, or Cys;  
 Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

wherein from 4 to about 44 of the amino acids designated by  
 Xaa are different from the corresponding amino acids of native  
 35 (1-133) human interleukin-3; with the proviso that no more  
 than one of the amino acids at positions 63, 82, 87, 98, 112  
 and 121 are different from the corresponding amino acids in  
 native human interleukin-3; wherein from 1 to 14 amino acids  
 are optionally deleted from the N-terminus and/or from 1 to 15

amino acids are optionally deleted from the C-terminus of said interleukin-3 mutant polypeptide; and wherein said interleukin-3 mutant polypeptide has increased activity relative to native human interleukin-3, in at least one assay  
 5 selected from the group consisting of: AML cell proliferation, TF-1 cell proliferation and Methylcellulose assay; and

(ii) a polypeptide comprising an N-terminal methionine residue, alanine residue or methionine-alanine di-  
 10 peptide immediately preceding said sequence according to (i); and

(b) harvesting said cultured stem cells.

15 28. Cultured stem cells obtained from a method of ex vivo expansion of stem cells, comprising the steps of;

(a) culturing stem cells with a growth medium comprising a human interleukin-3 mutant polypeptide selected from the  
 20 group consisting of:

(i) the human interleukin-3 mutant polypeptide of [SEQ ID NO:19];

25 wherein

Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

30 Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;

Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val, or Gly;

Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe,  
 35 Ser, or Arg;

Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

- Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;  
 Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;  
 Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;  
 Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;  
 5 Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;  
 Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;  
 Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;  
 Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;  
 10 Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;  
 Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;  
 Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;  
 Xaa at position 22 is Asp, Leu, or Val;  
 15 Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;  
 Xaa at position 24 is Asn, or Ala;  
 Xaa at position 26 is Leu, Trp, or Arg;  
 Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;  
 Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr, Leu, Val, Glu, Phe, Tyr, Ile or Met;  
 20 Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;  
 Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Asn, Gln, Ala or Pro;  
 25 Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Asp, Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;  
 Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;  
 Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;  
 30 Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;  
 Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;  
 Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;  
 35 Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;  
 Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;  
 Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, Met, or;  
 Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;  
 40 Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;



- Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,  
Thr, Ala, Tyr, Phe, Leu, Val or Lys;
- Xaa at position 43 is Asn or Gly;
- Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
- 5 Xaa at position 45 is Glu, Tyr, His, Leu, Pro, or Arg;
- Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;
- Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
- Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
- Xaa at position 49 is Arg, Tyr, Trp, Ser, His, Pro, or Val;
- 10 Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;
- Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;
- Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
- Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or  
His;
- 15 Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
- Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or  
Leu;
- Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;
- Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,  
Trp, or Asn;
- 20 Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
- Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
- Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
- Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,  
Gln, or Leu;
- 25 Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or  
Asp;
- Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;
- Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
- 30 Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or  
Asp;
- Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
- Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
- Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,  
His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
- 35 Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;
- Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;
- Xaa at position 71 is Leu, Asn, Val, or Gln;
- Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;
- 40 Xaa at position 73 is Leu, Ser, Trp, or Gly;
- Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;



- Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
- Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;
- Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
- 5 Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;
- Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
- Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala or Pro;
- 10 Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile or Tyr;
- Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;
- Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;
- Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Tyr or Pro;
- 15 Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
- Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, Pro;
- 20 Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;
- Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
- Xaa at position 89 is Asp, or Ser;
- Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
- 25 Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
- Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
- Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala, or Pro;
- 30 Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;
- Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg, His, Glu, Ser, Ala or Trp;
- Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;
- 35 Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;
- Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp, Lys, Leu, Ile, Val or Asn;
- Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;
- Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr, Trp, or Met;
- 40 Xaa at position 102 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu,

Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;  
 Xaa at position 103 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;  
 Xaa at position 104 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;  
 Xaa at position 105 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;  
 5 Xaa at position 106 is Asn, Ala, Pro, Leu, His, Val, or Gln;  
 Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or  
 Gly;  
 Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,  
 Ile, Tyr, or Cys;  
 10 Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

wherein from 4 to about 44 of the amino acids designated by  
 Xaa are different from the corresponding amino acids of native  
 (1-133) human interleukin-3; with the proviso that no more  
 15 than one of the amino acids at positions 63, 82, 87, 98, 112  
 and 121 are different from the corresponding amino acids in  
 native human interleukin-3; and wherein said interleukin-3  
 mutant polypeptide has increased activity relative to native  
 human interleukin-3, in at least one assay selected from the  
 20 group consisting of: AML cell proliferation, TF-1 cell  
 proliferation and Methylcellulose assay; and

(ii) a polypeptide comprising an N-terminal  
 methionine residue, alanine residue or methionine-alanine di-  
 25 peptide immediately preceding said sequence according to (i);  
 and

(b) harvesting said cultured stem cells.

30 29. Cultured stem cells obtained from a method of ex vivo  
 expansion of stem cells, comprising the steps of;

(a) culturing said stem cells with a growth medium  
 comprising a human interleukin-3 mutant polypeptide of (SEQ ID  
 35 NO:129);

wherein Xaa at position 18 is Asn or Ile; Xaa at position 19  
 is Met, Ala or Ile; Xaa at position 20 is Ile, Pro or Leu; Xaa  
 at position 23 is Ile, Ala or Leu; Xaa at position 25 is Thr  
 or His; Xaa at position 29 is Gln, Arg, Val or Leu; Xaa at  
 5 position 32 is Leu, Ala, Asn or Arg; Xaa at position 34 is Leu  
 or Ser; Xaa at position 37 is Phe, Pro, or Ser; Xaa at  
 position 38 is Asn or Ala; Xaa at position 42 is Gly, Ala,  
 Ser, Asp or Asn; Xaa at position 45 is Gln, Val, or Met; Xaa  
 at position 46 is Asp or Ser; Xaa at position 49 is Met, Ile,  
 10 Leu or Asp; Xaa at position 50 is Glu or Asp; Xaa at position  
 51 is Asn Arg or Ser; Xaa at position 55 is Arg, Leu, or Thr;  
 Xaa at position 56 is Pro or Ser; Xaa at position 59 is Glu or  
 Leu; Xaa at position 60 is Ala or Ser; Xaa at position 62 is  
 Asn, Val or Pro; Xaa at position 63 is Arg or His; Xaa at  
 15 position 65 is Val or Ser; Xaa at position 67 is Ser, Asn, His  
 or Gly; Xaa at position 69 is Gln or Glu; Xaa at position 73  
 is Ala or Gly; Xaa at position 76 is Ser, Ala or Pro; Xaa at  
 position 79 is Lys, Arg or Ser; Xaa at position 82 is Leu,  
 Glu, Val or Trp; Xaa at position 85 is Leu or Val; Xaa at  
 20 position 87 is Leu, Ser, or Trp; Xaa at position 88 is Ala or  
 Trp; Xaa at position 91 is Ala or Pro; Xaa at position 93 is  
 Pro or Ser; Xaa at position 95 is His or Thr; Xaa at position  
 98 is His, Ile, or Thr; Xaa at position 100 is Lys or Arg; Xaa  
 at position 101 is Asp, Ala or Met; Xaa at position 105 is Asn  
 25 or Gln; Xaa at position 109 is Arg, Glu or Leu; Xaa at  
 position 112 is Thr or Gln; Xaa at position 116 is Lys, Val,  
 Trp or Ser; Xaa at position 117 is Thr or Ser; Xaa at position  
 120 is Asn, Gln, or His; Xaa at position 123 is Ala or Glu;  
 with the proviso that from four to about forty-four of the  
 30 amino acids designated by Xaa are different from the  
 corresponding amino acids of native human interleukin-3 and  
 with the proviso that the human interleukin-3 mutant  
 polypeptide has increased activity relative to native human  
 interleukin-3, in at least one assay selected from the group

consisting of: AML cell proliferation, TF-1 cell proliferation and Methylcellulose assay; and

(b) harvesting said cultured stem cells.

5 30. Cultured stem cells obtained from a method of ex vivo expansion of stem cells comprising the steps of;

(a) culturing said stem cells with a growth medium comprising a human interleukin-3 mutant polypeptide of (SEQ ID  
10 NO:130);

wherein; Xaa at position 4 is Asn or Ile; Xaa at position 5 is Met, Ala or Ile; Xaa at position 6 is Ile, Pro or Leu; Xaa at position 9 is Ile, Ala or Leu; Xaa at position 11 is Thr or  
15 His; Xaa at position 15 is Gln, Arg, Val or Leu; Xaa at position 18 is Leu, Ala, Asn or Arg; Xaa at position 20 is Leu or Ser; Xaa at position 23 is Phe, Pro, or Ser; Xaa at position 24 is Asn or Ala; Xaa at position 28 is Gly, Ala, Ser, Asp or Asn; Xaa at position 31 is Gln, Val, or Met; Xaa  
20 at position 32 is Asp or Ser; Xaa at position 35 is Met, Ile, Leu or Asp; Xaa at position 36 is Glu or Asp; Xaa at position 37 is Asn, Arg or Ser; Xaa at position 41 is Arg, Leu, or Thr; Xaa at position 42 is Pro or Ser; Xaa at position 45 is Glu or Leu; Xaa at position 46 is Ala or Ser; Xaa at position 48 is  
25 Asn, Val or Pro; Xaa at position 49 is Arg or His; Xaa at position 51 is Val or Ser; Xaa at position 53 is Ser, Asn, His or Gly; Xaa at position 55 is Gln or Glu; Xaa at position 59 is Ala or Gly; Xaa at position 62 is Ser, Ala or Pro; Xaa at position 65 is Lys, Arg or Ser; Xaa at position 67 is Leu,  
30 Glu, or Val; Xaa at position 68 is Leu, Glu, Val or Trp; Xaa at position 71 is Leu or Val; Xaa at position 73 is Leu, Ser or Trp; Xaa at position 74 is Ala or Trp; Xaa at position 77 is Ala or Pro; Xaa at position 79 is Pro or Ser; Xaa at position 81 is His or Thr; Xaa at position 84 is His, Ile, or

Thr; Xaa at position 86 is Lys or Arg; Xaa at position 87 is Asp, Ala or Met; Xaa at position 91 is Asn or Gln; Xaa at position 95 is Arg, Glu, Leu; Xaa at position 98 Thr or Gln; Xaa at position 102 is Lys, Val, Trp or Ser; Xaa at position 103 is Thr or Ser; Xaa at position 106 is Asn, Gln, or His; Xaa at position 109 is Ala or Glu; with the proviso that from four to about forty-four of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3 and with the proviso that the human interleukin-3 mutant polypeptide has increased activity relative to native human interleukin-3, in at least one assay selected from the group consisting of: AML cell proliferation, TF-1 cell proliferation and Methylcellulose assay; and

(b) harvesting said cultured stem cells.

31. The cultured stem cells according to Claim 27 wherein said interleukin-3 mutant polypeptide is selected from the group consisting of:

(SEQ ID NO:66), (SEQ ID NO:67), (SEQ ID NO:68), (SEQ ID NO:69), (SEQ ID NO:70), (SEQ ID NO:71), (SEQ ID NO:72), (SEQ ID NO:73), (SEQ ID NO:74), (SEQ ID NO:75), (SEQ ID NO:76), (SEQ ID NO:77), (SEQ ID NO:78), (SEQ ID NO:79), (SEQ ID NO:80), (SEQ ID NO:81), (SEQ ID NO:82), (SEQ ID NO:83), (SEQ ID NO:84), (SEQ ID NO:85), (SEQ ID NO:86), (SEQ ID NO:87), (SEQ ID NO:88), (SEQ ID NO:89), (SEQ ID NO:90), (SEQ ID NO:91), (SEQ ID NO:92), (SEQ ID NO:93), (SEQ ID NO:94), (SEQ ID NO:95), (SEQ ID NO:96), (SEQ ID NO:296), (SEQ ID NO:300), (SEQ ID NO:301), (SEQ ID NO:308), (SEQ ID NO:309), (SEQ ID NO:310), (SEQ ID NO:315), (SEQ ID NO:316), and (SEQ ID NO:318).

32. The cultured stem cells according to Claim 27 wherein said mutant human interleukin-3 polypeptide has at

least three times greater activity than native human interleukin-3, in at least one assay selected from the group consisting of: AML cell proliferation, TF-1 cell proliferation and Methylcellulose assay.

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33. The cultured stem cells according to Claim 27 wherein said method further comprising the step of separating the stem cells from a mixed population of cells prior to culturing the stem cells.

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34. The cultured stem cells according to Claim 27 wherein said method said stem cells are separated from a mixed population of cells based on the stem cells having CD34 surface antigen.- -

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#### REMARKS

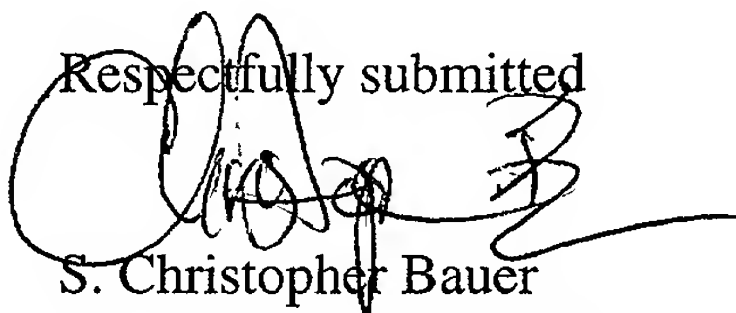
Support for new claims 27-34 is found in claim 8 as filed, which was subject to restriction in the parent application 08/764,114 (dated 06/26/98), and in the claims from which they depended. New claims 27-34 are the subject matter of claim 8 recast to include the limitations of the claims, from which they depended.

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Claims 1-26 have been canceled without prejudice. New claims 27-34 are pending. No new matter has been added.

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